

A Salt-Threshold Required for Exacerbating Insulin Resistance in Dahl Salt-Sensitive (S) Rats

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Abstract: The Dahl salt-sensitive (S) rat is a model of genetically determined salt-sensitivity and insulin resistance. In fact, insulin resistance is an inherited genetic trait that precedes and eventually co-exists with salt-sensitive hypertension in Dahl S, but not salt-resistant (R) Dahl rats. Owing to the critical role of salt in accelerating and exacerbating hypertension in Dahl S rats, researchers have investigated the role of salt in enhancing and/or sustaining insulin resistance in Dahl S rats. Conflicting data in the literature are available regarding the role of salt in insulin resistance in Dahl S rats. Therefore, this commentary is meant to address in depth some of the possible and relevant interpretations to some of the major findings published in the field of dietary salt contribution to insulin resistance. A conclusion has been drawn to provide a possible salt-threshold that is required for exacerbating insulin resistance in Dahl S rats.

Commentary

Insulin resistance and/or hyperinsulinemia are genetically determined in Dahl salt-sensitive (S), but not Dahl salt-resistant (R) rats. Weanling Dahl S, but not Dahl R rats are insulin resistant prior to developing spontaneous hypertension, which takes approximately 2–3 months to develop in Dahl S rats (Somova, Channa, 1999), and prior to salt loading, which takes 4 weeks of 8% NaCl to stabilize hypertension in Dahl S rats (Sechi et al. 1997).

On the other hand, fully developed hypertensive Dahl S rats remain insulin resistant as evidenced by their i) higher plasma insulin levels (Ogihara et al. 2002, Reaven, Twersky and Chang, 1991b; Somova, Channa, 1999), ii) defective insulin-stimulated glucose uptake by adipocytes (Reaven, Twersky and Chang, 1991a; Reaven, Twersky and Chang, 1991b), iii) higher plasma triglyceride concentrations (Reaven, Twersky and Chang, 1991b), iv) decreased glucose infusion rate, clearance, and utilization rate, and v) higher hepatic glucose production as assessed by the hyperinsulinemic euglycemic clamp procedure (Ogihara et al. 2002; Somova, Channa, 1999).

Owing to the dual presence of insulin resistance and salt-sensitivity of blood pressure in Dahl S rats, it has been hypothesized that salt which triggers hypertension in this model might be a potential contributor to an enhanced and/or sustained insulin resistance in fully hypertensive Dahl S rats. Sodium loading in Dahl S rats at the following concentrations and for the following durations (3% NaCl for 1 week (Kotchen et al. 1991), or 8% NaCl for 2 weeks (Reaven, Twersky and Chang, 1991a), or 2% NaCl for 2 months (Somova, Channa, 1999) maintained, but did not further increase their insulin resistance. However, only when Dahl S rats were fed a diet containing 8% NaCl for 4 weeks did they show significant insulin resistance compared to Dahl S rats fed 0.3% NaCl for 4 weeks (Ogihara et al. 2002). Enhanced insulin resistance in Dahl S rats fed 8% NaCl for 4 weeks is manifested by a marked suppression in the glucose infusion rate, utilization rate and glucose uptake by the muscles (Ogihara et al. 2002). Enhanced insulin resistance in Dahl S rats fed dietary 8% NaCl for 4 weeks was concomitant with an enhanced and generalized oxidative stress response (Swei et al. 1997) and an inflammatory response (Hamaguchi et al. 2000). Therefore, 8% NaCl for 4 weeks appears to be the threshold for an enhanced insulin resistance (Ogihara et al. 2002), oxidative stress (Swei et al. 1997), inflammation (Hamaguchi et al. 2000) and stable hypertension in Dahl S rats (Sechi et al. 1997).

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Ogihara and co-authors in 2002 have assessed the impact of 4 weeks of dietary 8% NaCl on insulin resistance in Dahl S rats using the hyperinsulinemic euglycemic clamp procedure which is a sensitive technique and yields reliable and reproducible results. The hyperinsulinemic euglycemic clamp procedure demonstrated clearly the insulin resistance in Dahl S rats fed 8% NaCl *versus* 0.3% NaCl by a significant decrease in the glucose infusion rate, utilization rate and uptake by muscles. Then, they proceeded by fasting the rats for 12 hours, after which immediately they harvested liver, muscle and fat tissues for immunoprecipitation and immunoblotting experiments and to test for the phosphoinositide-3 kinase (PI3K) activity and protein kinase B (Akt) serine phosphorylation. A significant activation in the insulin signaling pathway manifested by an enhanced tyrosine phosphorylation of the insulin receptor, insulin receptor substrates, activation of phosphoinositide-3 kinase (PI3K) and phosphorylation of Akt was observed in Dahl S rats fed 8% NaCl for 4 weeks compared to Dahl S rats fed a low salt diet (0.3% NaCl) for 4 weeks. While it is tempting to conclude that high salt exacerbates insulin resistance through defects along the insulin signaling pathway downstream the PI3K and possibly at the level of glucose transport and/or phosphorylation, we need to underscore the impact of fasting in Dahl S model of insulin resistance. While fasting might be beneficial in getting accurate readings for fasting plasma insulin and glucose measurements, fasting plays a significant role in insulin resistance. It is well documented that 12-hours of fasting upregulated hepatic insulin receptors (Seymour, Volpert and Andersen, 1996), enhanced tyrosine phosphorylation of the insulin receptor, insulin receptor substrates, activated PI3K and phosphorylated Akt in insulin resistant rat models compared to their fed controls (Rojas, Hirata and Saad, 2001). These effects of fasting in insulin resistant models are identical to those observed by Ogihara et al. in 2002 in starved Dahl S rats. Interestingly, Dahl S rats had a significant weight loss after 4 weeks of high salt diet (8% NaCl) compared to normal salt diet and to Dahl R controls (Hamaguchi et al. 2000; Ogihara et al. 2002; Ogihara, Asano and Fujita, 2003). It is known that fasting significantly enhances insulin signaling in lean *versus* obese subjects with similar effects to those observed in Dahl S rats after 12 hours of starvation (Bergman et al. 2007). In agreement with the role of fasting

in enhancing the insulin signaling pathway in Dahl S rats despite the hidden defects that might be present in the insulin signaling pathway is the fact that fasting leveled off the higher plasma insulin levels between Dahl S and R rats, which should have been significantly higher in Dahl S *versus* R rats. Moreover, the muscle glucose transporter 4 (GLUT4) levels were comparable in Dahl S and R rats, when one would have expected an increase in GLUT4 in Dahl S *versus* R rats. GLUT 4 mRNA synthesis (Garcia de Herreros, Birnbaum, 1989) and protein expression (Ciaraldi et al. 1995) are greatly dependent on insulin and therefore hyperinsulinemia in Dahl S rats would suggest an increased GLUT4 in this model of insulin resistance.

In conclusion, we do support the role of dietary 8% NaCl when given for at least 4 weeks in enhancing insulin resistance in Dahl S rats. This was clearly shown by results from the hyperinsulinemic euglycemic clamp procedure performed on anaesthetized rats. However, the cellular and molecular mechanism of insulin resistance in Dahl S rats demonstrated by an overall activation of the insulin signaling pathway might be the consequence of 12-hours of fasting in this model of insulin resistance. Clearly, there is much research to be performed before we fully understand how insulin resistance occurs in this Dahl genetic model of salt-sensitivity and insulin resistance. As recently hypothesized, insulin resistance in Dahl S rats might still be mediated via an impaired insulin signaling, or possibly genetic mutation (s) in the insulin receptor substrates 1 and/or 2 that might hinder their association with downstream molecules in the insulin signaling pathway and prevent glucose uptake by the cells (Shehata, 2008a; Shehata, 2008b).

Disclosure

The authors report no conflicts of interest.

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